Please enter the following new claims:

--15. A method of screening for a bioactive agent capable of altering a cellular phenotype, said method comprising:

a) combining at least one candidate bioactive agent and of a population of cells;

b) sorting said cells in a FACS machine by separating said cells on the basis of at least five cellular parameters which allow detection of alterations in cellular phenotype, whereby cells with altered cellular phenotype are identified and said alteration in cellular phenotype indicates said candidate is a bioactive agent capable of altering a cellular phenotype ; and

c) repeating steps a) and b) with a different candidate bioactive agent.

16. The method of any one of Claims 1-7, 10-13 and 15, wherein measurement of each of said cellular parameters is done approximately simultaneously.--

REMARKS

Claims 1-16 are presented for prosecution. The present claims are provided in an attached appendix for the Examiner's convenience.

Claims 1 and 3 have been amended for clarity. Support is found throughout the specification, for example at page 8, lines 12-18.

Claim 15 is added to claim a method of screening for a bioactive agent capable of altering a cellular phenotype. Support is found, for example, in original Claim 1, at page 32, lines 9-11, and at page 51, lines 27-29.

Claim 16 is added as a claim depending from claims 1-7, 10-13 and 15, specifying that measurement of each of the cellular parameters is done approximately simultaneously. Support is found, for example, at page 32, lines 6-7.

Objection to Finality of Office Action

The Office Action contains new rejections which were not necessitated by Applicants amendments. Specifically, Claims 1-7 were previously rejected under 35 U.S.C. § 102(a) over Nolan, WO 97/27212 (Nolan). The same rejection in the current Office Acton encompasses Claim 1-4, 7-10 and 13-14. It is not clear how Applicants amendment, particularly of Claims 8-10, which were only amended (directly or indirectly) as to dependence, no new independent claims were added, and amendments to the independent claims only served to clarify the claims and did not broaden them. The new rejection of at least Claims 8-10 and 14 (which depends from Claim 8) finds no basis in the Applicants' amendment.

Similarly, Claim 3 was previously rejected under 35 U.S.C. § 102(e) over Kamb, USPN 5,955,275 (Kamb). The same rejection in the current Office Action encompasses Claims 3, 8, 10 and 14. As discussed above, the claims were not broadened.

In addition, Claims 5-6 were previously rejected under 35 U.S.C. § 103(a) over Nolan or Kamb in view of Hide et al., *J. Cell Biol.* 123(30:585-593 (1993) (Hide). The same rejection in the present Office Action encompasses Claims 5-6 and 11-12. Claims 11 and 12, added in the response to the previous Office Action, depend (directly or indirectly) from Claim 4, not Claim 5 or Claim 6.

The Office Action makes no showing as to how Applicants amendments necessitated these new rejections. Applicants submit that the new rejections were not necessitated by Applicants' amendments and respectfully request withdrawal of finality of the Office Action or, in the Alternative: withdrawal of the rejection over Nolan for Claims 8-10, 13 and 14; withdrawal of the rejection over Kamb for Claims 8, 10 and 14; and withdrawal of the rejection over Nolan or Kamb in view of Hide for Claims 11 and 12.

35 U.S.C. § 112 Rejections

Claims 1-7 are rejected under 35 U.S.C. § 112, first paragraph as not being enabled by the specification. Applicants respectfully traverse.

The test for enablement under 35 U.S.C. § 112, first paragraph, is whether a claim is supported by the disclosure such that one of ordinary skill in the art could make and use the invention at the time the application was filed (*see* MPEP § 2164). Applicants submit that the present methods are enabled for use with a multitude of candidate bioactive agents and various cell types, as disclosed in the specification.

The Office Action suggests that the present methods are not enabled because the specification does not provide enablement unless a library of bioactive agents are used in the present methods. Applicants respectfully point out that if a single candidate agent which, in fact, is capable of altering the cellular phenotype were combined with a population of cells and sorted as claimed, such a candidate agent would have been screened for and identified without a library of candidate agents.

Claim 1 has been amended to recite a method for screening a population of cells for at least one cell with an altered cellular phenotype. The method involves combining a candidate bioactive agent and a population of cells and sorting the cells to identify an altered phenotype. Clearly, the candidate agent cannot be limiting, as virtually any agent (e.g., any agent of the type listed on page 16, lines 8-10 of the specification) may be combined with a population of cells, as claimed, and alteration of phenotype by FACS sorting, as claimed, can be identified.

A feature of the claimed methods is the use of multiple cellular parameters (at least three or at least five) to screen for a bioactive agent capable of altering a cellular phenotype. That this is enabled is not questioned. The examples provided evidence that many different types of cells may be assayed in this way. The use of FACS sorting identifies cells with altered cellular phenotype, which, if such cells are identified, indicates that the bioactive agent combined with the cells is capable of such alteration.

The Office Action mailed October 6, 1999 (Paper 6) questions how the example of determining the effects on the cellular phenotype of cells transformed with "a single bioactive agent, p21" (page 6) supports screening a multitude of nucleic acids transfected into a population of cells (referring to Claim 3). Applicants point out that the example does not assay the effects of one, but of three agents, the p21, a PCNA binding C-terminus fragment of p21, and a C-terminus fragment mutant that does not bind PCNA. Cells transfected with the nucleic acid encoding p21 or PCNA binding fragment were shown to have altered cellular phenotype, while that transfected with the mutant fragment did not. This is presented as a simple example of positive and negative result.

Applicants do not see the "incalculable parameters included in the broadly claimed scope" to which the Office Action refers. The ordinary skilled artisan would understand that the screening of a given library would be in the same cell type under the same conditions such that the parameters that the cells face are the same except for the nucleic acid with which each is transfected, and the same parameters which allow detection of alteration in cellular phenotype would be used. The only variable is the agent (nucleic acid or protein encoded thereby). Cells whose phenotype have been altered are identified by sorting using FACS. It is well within the skill of the ordinary artisan to determine with which agent (nucleic acid or protein product thereof) the cells with altered phenotype have been transfected.

Applicants point out, again, that the activity of the candidate agent, vis-a-vis ability to alter cellular phenotype, need not be known prior to the screening. To identify agents having no known activity regarding ability to alter cellular phenotype is precisely what the screening is for. The positive result unequivocally identifies an agent with the capability tested for, regardless of the conformation of the resultant product.

For the reasons discussed above, Claims 1-7 satisfy the enablement requirements of 35 U.S.C. § 112, first paragraph. Therefore, the Examiner is respectfully requested to withdraw this rejection.

Provisional Rejections

Claims 1-3 and 5-6 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-6, 12-33 and 35 of copending Application No. 09/062,330. Claims 1-7 are provisionally rejected under the same doctrine as being unpatentable over claims 1-3 and 5 of copending Application No. 09/157,748.

Applicants request that the Examiner hold these rejections in abeyance until such time as otherwise patentable subject matter is found.

Claims 1-7 are provisionally rejected under 35 U.S.C. § 103(a) as being obvious over copending Application No. 09/157,748. Applicants respectfully traverse.

Applicants respectfully request that this provisional rejection be held in abeyance. Applicants are considering submission of a declaration under 37 C.F.R. §1.132 to show that relevant disclosure in the present application is the work of the inventor in common in the two applications or filing of a CPA, thus bringing the application under the new provisions of 35 U.S.C. §103(c) and making the rejection inoperable.

35 U.S.C. § 102 Rejections

Claims 1-4 and 7-10 and 13-14 are rejected under 35 U.S.C. § 102(a) as being anticipated by Nolan. Applicants respectfully traverse.

For a reference to anticipate a claim, it must teach each and every element of the claim (*see* MPEP § 2131 and references cited therein). The present claims are not anticipated by the disclosure of Nolan because Nolan does not explicitly teach sorting cells in a FACS machine by separating cells on the basis of at least five (Claims 1-2) or at least three (Claims 3-4 and 7) cellular parameters.

Cellular parameters are aspects of the cellular phenotype which are measured, as the specification teaches (*see, e.g.*, page 8, lines 15-17). A distinguishing feature of all of

the rejected claims is that the claims call for sorting cells in a FACS machine by separating the cells on the basis of at least five or at least 3 cellular parameters. A person of ordinary skill in the art would understand this phrase to mean that such measurements, at least 5 or at least 3 of these measurements, are made in the FACS machine and the cells are sorted on the basis of these at least 5 or at least 3 measurements.

The Office Action cites several passages from the Nolan reference to support this rejection. The majority of these passages are not related to the sorting of cells in a FACS machine. For example, no relationship is shown between the list of phenotypic changes provided on page 31, lines 7-26 that might be measured and sorting of cells in a FACS machine. The Office Action cites phenotypic parameters such as increased cell death or cell viability, but such parameters are not explicitly taught to be measured in a FACS machine. Dye staining techniques, also cited in the Office Action as measurement of a cellular parameter, are clearly offered in the alternative to FACS. The Office Action suggests that measurement of the presence of a cell would constitute a cellular phenotype parameter, but one of ordinary skill in the art would not. Applicants submit that this passage does not explicitly teach measuring at least 3 or at least 5 cellular parameters in a FACS machine.

The Office Action also enumerates isolation techniques cited on page 33, lines 19-28 of Nolan. This passage recites some isolation techniques which rely on the identification a single cellular phenotype parameter (e.g., cell death or expression of an induced cell surface protein). However, the passage the different techniques are presented in the alternative to each other. More importantly, the techniques are presented in the alternative to using FACS.

Finally, the Office Action cites Example 1, wherein FACS is used to sort cells based on an Apotag assay (i.e., the single parameter of apoptosis) or in an other example, by propidium iodine staining as a measure of a single cellular parameter (apoptosis). The use of ethidium bromide/acridine orange staining is not taught in conjunction with FACS.

Therfore, the Office Action has not shown where Nolan explicitly teaches measuring at least 3 or at least 5 cellular parameters in a FACS machine.

A skilled artisan would understand from the language of the claims that the methods call for sorting using a FACS machine, such sorting in the FACS machine being based on at least five or at least three cellular parameters. Nolan does not teach or suggest this feature of the claims. Therefore, Applicants respectfully request withdrawal of this 35 U.S.C. § 102(a) rejection of claims 1-4, 7-10 and 13-14.

Claims 3, 8, 10 and 14 are rejected under 35 U.S.C. § 102(e) as being anticipated by Kamb. Applicants respectfully traverse.

Applicants reiterate that Claim 3 has the element of sorting cells in a FACS machine by separating cells on the basis of at least three cellular parameters. However, Kamb teaches the separation of cells based on a single reporter gene.

Kamb discloses sorting a population of cells based on a single reporter gene. In one example, (example 1), a population of cells is sorted based on the presence of an antibody. This population is not sorted on any other basis. In another example, the population of cells is sorted based on expression of a reporter gene. this population is not sorted based on any other basis. Nowhere does Kamb disclose or suggest sorting of a population of cells in a FACS machine on the basis of at least three cellular parameters. An alternative reporter gene is disclosed, however, sorting of a population of cells on the basis of expression of more than one gene or more than one parameter is not. Contrary to the assertion in the Office Action, GFP is an expressed reporter gene, not a "vital dye" that is taken up by living (as opposed to dead) cells. Kambs reference to GFP as a "vital dye" is actually referring to the fact that the GFP is expressed without killing the cell. GFP and BFP are fluorescent polypeptides. The use of one reporter gene to sort one population and another reporter gene to sort another population would not be construed by the skilled artisan as being the same, or even suggesting, sorting a population of cells

on the basis more than one cellular parameter. Neither does reference to the use of GFP "or other modes of light emission" suggest the sorting of a population of cells on the basis of more than one parameter. Alternatives for one is not the same as, nor does it suggest, the use of at least three.

Based on the discussion above, Claims 3, 8, 10 and 14 are not anticipated by Kamb. Therefore, the Examiner is respectfully requested to withdraw this 35 U.S.C. § 102(e) rejection.

35 U.S.C. § 103 Rejection

Claims 5-6 and 11-12 are rejected under 35 U.S.C. § 103(a) as being obvious over either Nolan or Kamb in view of Hide et al., J. Cell Biol. 123(3):585-93 (1993) (Hide). Applicants respectfully traverse.

For a rejection under 35 U.S.C. 103 to be proper, the cited references must: 1) teach each of the elements of the rejected claim; 2) provide motivation to combine the teachings of the cited references; and 3) provide a reasonable expectation of success in obtaining the invention if such reference disclosures were combined. None of the cited references, alone or in any combination, teach all of the claim elements of the rejected claims.

As discussed above, neither Nolan nor Kamb teach or suggest all of the elements of Claim 3, from which Claims 5 and 6 depend. Hide et al. does not cure this shortcoming because this reference also does not disclose or suggest sorting cells in a FACS machine by separating cells on the basis of at least three cellular parameters. None of these references suggest how the refractile measure shown by Hide would be applied to sorting of cells in a FACS machine. None of these references suggest sorting of a population of cells on the basis of at least three cellular parameters. No motivation to combine the references is found in any of the references, either taken alone or in

conjunction; none of the references consider sorting of a population of cells on the basis of multiple parameters.

The Office Action makes the assertion at the end of this rejection that, "It has been well known in the art that combinations of these different parameters using FACS machines would lead to a clearer identification of the cellular phenotype alterations." However, no evidence to support this assertion is presented.

Based on the above discussion, a *prima facie* case for obviousness of Claims 5-6 and 11-12 over Nolan or Kamb in view of Hide has not been shown. Therefore, the Examiner is respectfully requested to withdraw this 35 U.S.C. § 103(a) rejection.

Applicants submit that the Claims are now in form for allowance and earnestly request such a finding. If after review of this response, the Examiner has further unresolved issues, the Examiner is respectfully requested to phone the undersigned at (415) 781-1989.

Respectfully submitted,

FLEHR HOHBACH TEST
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Dated: 5 Jan., 2001

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APPENDIX

1. (Twice Amended) A method of screening [for a bioactive agent capable of altering] a population of cells for at least one cell with an altered cellular phenotype, said method comprising:

a) combining at least one candidate bioactive agent and a population of cells; and

b) sorting said cells in a FACS machine by separating said cells on the basis of at least five cellular parameters which allow detection of alterations in cellular phenotype, whereby cells with altered cellular phenotype are identified [said ~~alteration in cellular phenotype indicates said candidate is a bioactive agent~~ capable of altering a cellular phenotype].

2. A method according to claim 1 wherein a library of candidate bioactive agents are combined with said population.

3. (Amended) A method of screening [for a bioactive agent capable of altering] a population of cells for at least one cell with an altered cellular phenotype, said method comprising:

a) introducing a library of nucleic acids each encoding a candidate bioactive agent into a population of cells; and

b) sorting said cells in a FACS machine by separating said cells on the basis of at least three cellular parameters which allow detection of alterations in cellular phenotype, whereby cells with altered cellular phenotype are identified [said alteration in cellular phenotype indicates said candidate is a bioactive agent capable of altering a cellular phenotype].

4. A method according to claim 3 wherein said library is a retroviral library.

5. (Amended) A method according to claim 3 wherein said cellular phenotype is exocytosis and said cellular parameters are selected from the group consisting of light scattering, fluorescent dye uptake, fluorescent dye release, annexin granule binding, surface granule enzyme activity, and the quantity of granule specific proteins.

6. A method according to claim 5 further comprising subjecting said cells to conditions that normally cause exocytosis.

7. (Amended) A method according to claim 3 wherein said cellular phenotype is cell cycle regulation and said cellular parameters comprise cell viability, proliferation, and cell phase.

8. (Amended) A method according to claim 3, 4, 5, 6, 7, 11, 12 or 13 wherein said nucleic acids comprise fusion nucleic acids comprising:

a) said nucleic acid encoding said candidate bioactive agents; and

b) a detectable moiety.

9. (Amended) A method according to claim 1 or 2 wherein said cells are tumor cells.

10. A method according to claim 8 wherein said detectable moiety is a fluorescent protein.

11. A method according to claim 4 wherein said cellular phenotype is exocytosis and said cellular parameters are selected from the group consisting of light scattering, fluorescent dye uptake, fluorescent dye release, annexin granule binding, surface granule enzyme activity, and the quantity of granule specific proteins.

12. A method according to claim 11 further comprising subjecting said cells to conditions that normally cause exocytosis.

13. A method according to claim 4 wherein said cellular phenotype is cell cycle regulation and said cellular parameters comprise cell viability, proliferation, and cell phase.

14. A method according to claim 8 wherein said cells are tumor cells.

15. A method of screening for a bioactive agent capable of altering a cellular phenotype, said method comprising:

a) combining at least one candidate bioactive agent and of a population of cells;

b) sorting said cells in a FACS machine by separating said cells on the basis of at least five cellular parameters which allow detection of alterations in cellular phenotype, whereby cells with altered cellular phenotype are identified and said alteration in cellular phenotype indicates said candidate is a bioactive agent capable of altering a cellular phenotype ; and

c) optionally, repeating steps a) and b) with a different candidate bioactive agent.

16. The method of any one of Claims 1-7, 10-13 and 15, wherein measurement of each of said cellular parameters is done approximately simultaneously.